

Predicted NT: SEQ ID NO:2 GGG AGG GGC AAG AUC GAG AUC AAG CGC AUC GAG

Maize SEQ ID NO:3 GGG AGa GGC AAG AUC GAG AUC AAG CGC AUC GAG 32/33

Rice SEQ ID NO:4 GGG AGG GGg AAG AUC GAG AUC AAG CGg AUC GAG 31/33

Arabidopsis SEQ ID NO:5 GGG AGA GGA AAG AUC GAA AUC AAA CGG AUC GAG (M) 28/33
(R) 29/33

(B)

GENE: APETALA1
FUNCTION: TRANSCRIPTION FACTOR
DOMAIN: MADS BOX

AA SEQUENCE: SEQ ID NO:6 R I E N K I N R Q V T F

Predicted NT: SEQ ID NO:7 AGG AUC GAG AAC AAG AUC AAC AAG CAG GUG ACC UUC

Maize SEQ ID NO:8 cGG AUC GAG AAC AAG AUC AAC cGG CAG GUg ACC UUC 33/36

Rice SEQ ID NO:9 AGG AUC GAG AAC AAG AUC AAC cGG CAG GUG ACg UUC 34/36

Arabidopsis SEQ ID NO:10 AGG AUA GAG AAC AAG AUC AAA AGA CAA GUG ACA UUC (M)29/36
(R)30/36

(C)

GENE: APETALA2
FUNCTION: TRANSCRIPTION FACTOR
DOMAIN: AP2 DOMAIN

AA SEQUENCE: SEQ ID NO:11 G R W E S H I W D C

Predicted NT: SEQ ID NO:12 GGC AGG UGG GAG UCC CAC AUC UGG GAC UGC

Maize SEQ ID NO:13 GGC cGc UGG GAa UCC CAC AUC UGG GAC UGC 27/30

Arabidopsis SEQ ID NO:14 GGA AGA UGG GAA UCU CAU AUU UGG GAC UGU (M) 23/30

Example 3: SPECIFICITY OF CODON ADJUSTED PRIMERS

The following example illustrates the specificity of codon adjusted primer pairs. Primers 1 and 2 represent primers taken directly from the sequence of the template polynucleotide. Primers 1' and 2' are primers wherein the sequence has been codon adjusted for monocots according to the invention. These primers were used to identify target polynucleotides from corn and rice.

Primer 1

AA SEQUENCE SEQ ID NO:15 D C G L Q V

Coding Sequence: SEQ ID NO:16 5' G GAC TGT GGG AAA CAA GTT TA 3'

Primer 1 Sequence: SEQ ID NO:17 5' G GAC TGT GGG AAA CAA GTT TA 3'

Primer 1' (Codon Adjusted Sequence): SEQ ID NO:18 5' G GAC TGC GGG AAG CAG GTG TA 3'
17/21

%Sequence Identity to Primer 1: 81%

Primer 2

AA SEQUENCE	SEQ ID NO:19	K Y R G V T L
Coding Sequence:	SEQ ID NO:20	5' AAG TAT AGA GGT GTC ACT TTG CA 3'
Complement	SEQ ID NO:21	3' TTC ATA TCT CCA CAG TGA AAC GT 5'

Primer 2 Sequence:	SEQ ID NO:22	5' TG CAA AGT GAC ACC TCT ATA CTT 3'
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Codon Adjusted Sequence:	SEQ ID NO:23	5' AAG TAC AGG GGC GTC ACC TTG CA 3'
Complement	SEQ ID NO:24	3' TTC ATG TCC CCG CAG TGG AAC GT 5'

Primer 2' Sequence:	SEQ ID NO:25	5' TG CAA GGT GAC GCC CCT GTA CTT 3' 19/23
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%Sequence Identity to Primer 2: 83%

PCR was performed as described in Example 1 using genomic DNA from *Arabidopsis thaliana*, *Oryza sativa* (rice) and *Zea mays* (corn) as a source for the desired target polynucleotides.

RESULTS AND CONCLUSIONS:

PCR-amplified products of the expected size were generated using primers 1 and 2 and *Arabidopsis* genomic DNA as a substrate. No products were obtained in reactions using either rice or corn genomic DNA substrate.

On the other hand, PCR-amplified products were generated using the codon adjusted primers 1' and 2' and corn genomic DNA as a substrate. No products were obtained in a reaction using *Arabidopsis* genomic DNA substrate. Together, these results demonstrate the general utility of designing codon adjusted primers for use in isolating/identifying gene orthologs from different plant families.

Example 4

The method of the invention was used to isolate AP2-like genes from *Avena sativa* (oat), *Oryza sativa* (rice), *Triticum aestivum* (wheat) and *Zea mays* (corn). Primers 1' and 2' described in Example 3 were used in PCR using the conditions of Example 1 and genomic DNA

from each plant as a source of target polynucleotides. The nucleotide and corresponding amino acid sequences of PCR-amplified products are shown below.

>OAT ADC GENE 489 BP SEQ ID NO:26
TACCTAGGTGAGCTCAAATTCAGCTCCAGCTCCTCCTAATTAATTCCATCTGTTCTGTGTAAGTTATTTAATTTTCGTAGGTGGTTTCGACACCGCG
CACTCGGCGCGAGGTTATTAATTAATCAAGCTTCTAGTTGAACCTTTCAACACATACTGCTCTCTCGATTGGATTGTACTAGCATCATGAACGTACTGAA
ACGGGTCTTGTCTCAGGGCCTACGATCGCGCGCGGATCAAGTTCCGGGGACTGGACGCCGACATCAACTTCAATCTGAGCGACTACGAGGAGGATCTGAAGCAGG
TAACTGAATAAGATCGCTTCTCAAATGCAGCATAGATATTATCGGTGTGTGTGTCTGATGGGTGGTTGGTGGCCGGCCGGGCACTCTTGTTTTGGCAGAT
GAGGAACTGGACCAAGGAGGAGTTCTGTGCACATCTCCGCGCGCAGAGCACGGGTTCCGAGGGGGAGCTCA

>OAT ADC PROTEIN 65 aa SEQ ID NO:27
GGFDTAHSAARAYDRAAIKFRGLDADINFNLSYEDDLKQVTNWTKEEFVHILRRQSTGFARGSS

>RICE AP2-LIKE GENE 387 BP SEQ ID NO:28
CCTAGGTAATTTTCATCGAACACATCATCTTCTCTCTCAATCCAACGGACATCGCCATGAACAATCTAACAAACACCTTCATCTTCTCCAAACAATCACAG
GTGGATTTCGACACTGCTCAGCGAGCTGCAAGGTAAAGAACACATCACATCATTCATCAGAACATGAGCTCTGTGTTTGTGAAGGAGATTGAGAGAATTGAATGA
TGATGGATGGATCGAGGCGGTACGACAGGGCGCGGATCAAGTTCAAGGGAGTAGAGGCTGACATCAACTTCAACCTGAGCGACTACGAGGAGGACATGAGGCAG
ATGAAGAGCTTGTCCAAGGAGGAGTTCTGTGCACGTTCTCCGGCGACAGAGCACCGGCTTCTCCGCGGCGAGCTCA

>RICE ADC PROTEIN 65 aa SEQ ID NO:29
GGFDTAHAAARAYDRAAIKFRGVEADINFNLSYEDDMRQMSLSKEEFVHVLRRQSTGFSRGS

>WHEAT ADC GENE 477 BP SEQ ID NO:30
CTTGGGTGGGTTTGACACTGCACATGCTGCTGCAAGGTACGTACAATTTAATTAAGCACGTACGCACTACATAATTGTGATGTGATCATCACCTGAACACCT
GTACTGCAACTCTGAAGTTATGTCTCCACTCTGTTTCACTTCCCGTGCCTAATTTGACCTTGGGATGTTCCGCGAGGGCGTACGATCGAGCGCGCATCAAGTTCCG
CGGCGTCGACGCCGACATAAACTTCAACCTCAGCGACTACGAGGACGACATGAAGCAGGTGATCAGCAAAGCCACCAACAGTGTCTCTCATCCAACCAATTA
TTCAGATGCAGAGTGCATTAAGTGTGTTGAACTGATGAAGTGAAGAAATCTGACTGTGTGTTGTTGGTGGATGATCTGGATCAGATGAAGGGCCTGTC
CAAGGAGGAGTTCTGTGCACGTGCTGCGCGGCGAGCGCGGCTTCTCGCGGGGACAGCTC

>WHEAT ADC PROTEIN 65 aa SEQ ID NO:31
GGFDTAHAAARAYDRAAIKFRGVDADINFNLSYEDDMKQVGLSKKEEFVHVLRRQSTGFSRGS

>MAIZE ADC GENE 489 BP SEQ ID NO:32
CTTAGGTGAGCAGCAATAAGCAGATCGATCTGCAGCATAAATTTCCGTTATTAAGTCTGCTGATCTCGATCGAATGGCCTAATTAACCGATTTCGGTGTCT
GGCGGATGGCCAACTACGCGAGGTGGATTTCGACACTGCTCATGCCGTGCAAGGTAACGATCAATCCATCCACCCCTTGTCTAGCTACCCACCGACCGGC
CGGATTAATGGACCGCTAGTTCTCGGGACGGGCTTGTCTGAGGGCGTACGACCGAGCGCGGATCAAGTTCCGCGGGCGTACGCGCGACATAAACTTCAACCTCA
CGGACTACGACGACGATGAAGCAGGTACATACAGAGTGTGTTGACGCTAGCACCGACTGAAACATCTGCTGAACGTACACTCATGGCCTGTGCACAGAT
GAAGAGCCTGTCCAAGGAGGAGTTCTGTGCACGCTTCCGCGCGCAGAGCACCGGCTTCTCCGTTGGCAGCTCC

>MAIZE ADC PROTEIN 65 aa SEQ ID NO:33
GGFDTAHAAARAYDRAAIKFRGVDADINFNLSYEDDMKQVGLSKKEEFVHALRRQSTGFSRGS

EXAMPLE 5. USE OF SHORT CODON ADJUSTED PRIMERS

Oligonucleotides

Codon adjusted oligonucleotides were designed as described previously. Derivatives of oligonucleotide 2' were generated as shown above and used as primers in combination with oligonucleotide 1' in PCR reactions using plant genomic DNA from Zea mays (corn), Avena sativa (oat), and Triticum aestivum (wheat) as a source of target polynucleotides.

PCR

A typical PCR reaction consisted of 1-5 µg of target plant DNA, 10 pmol of primer 1' and 10 pmol of a derivative of primer 2', and 1.25 U of Taq DNA polymerase in standard 1X PCR reaction buffer as specified by the manufacturer (Promega, Madison, WI). PCR reaction conditions consisted of five cycles (5) of denaturation at 94oC for 2 minutes, 94oC for 30 sec., primer-template annealing at 65oC for 15 sec., 60oC for 15 sec., 55oC for 15 sec., 50oC for 15 sec., 45oC for 15 sec., 40oC for 15 sec., and synthesis at 68oC for 1 min., 30 sec., and twenty (20) cycles of denaturation at 94oC for 30 sec., primer-template annealing at 55oC for 30 sec., synthesis at 72oC for 1 min., 30 sec., thirty (30) cycles of denaturation at 94oC for 30 sec., primer-template annealing at 50oC for 30 sec., synthesis at 68oC for 1 min., followed by one (1) cycle of prolonged synthesis at 68oC for 7 min.

Primer 1

AA SEQUENCE	SEQ ID NO:34	D C G L Q V
Coding Sequence:	SEQ ID NO:35	5' G GAC TGT GGG AAA CAA GTT TA 3'
Primer Sequence:	SEQ ID NO:36	5' G GAC TGT GGG AAA CAA GTT TA 3'

Primer 1' (Codon Adjusted Sequence): SEQ ID NO:37 5' G GAC TGC GGG AAG CAG GTG TA 3'

Primer 2

AA SEQUENCE	SEQ ID NO:38	K Y R G V T L
Coding Sequence:	SEQ ID NO:39	5' AAG TAT AGA GGT GTC ACT TTG CA 3'
Complement	SEQ ID NO:40	3' TTC ATA TCT CCA CAG TGA AAC GT 5'

Primer 2 Sequence: SEQ ID NO:41 5' TG CAA AGT GAC ACC TCT ATA CTT 3'

Codon Adjusted Sequence:	SEQ ID NO:42	5' AAG TAC AGG GGC GTC ACC TTG CA 3'
Complement	SEQ ID NO:43	3' TTC ATG TCC CCG CAG TGG AAC GT 5'

Primer 2' Sequence: SEQ ID NO:44 5' TG CAA GGT GAC GCC CCT GTA CTT 3'

RISZU2'-1 (5 CODONS)	SEQ ID NO:45	5' G CAA GGT GAC GCC CCT GT 3'
RISZU2'-2 (5 CODONS)	SEQ ID NO:46	5' GGT GAC GCC CCT GTA CT 3'